

§Appl. No. 10/078,531
Amdt. dated August 25, 2004
Reply to Office Action of, May 27, 2004

In the Specification:

Please amend the specification as follows:

On page 2, the second full paragraph has been amended as follows:

The University of Oklahoma has set up a genome sequencing project for *S. pyogenes* strain M1 GAS (~~<http://dna1.chem.ou.edu/strep.html>~~).

On page 26, the first full paragraph has been amended as follows:

It was determined that the 3027-bp including a stop codon (TAA) open reading frame (ORF) of BVH-P7 encodes a 1008 amino-acid-residues polypeptide with a predicted pI of 6.18 and a predicted molecular mass of 111,494.44 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :2) using the PSORTII software (Real World Computing Partnership (~~<http://psort.nibb.ac.jp>~~)) suggested the existence of a 21 amino acid residues signal peptide (MKKHLKTVALTLTTVSVVTHN)(SEQ ID NO: 13), which ends with a cleavage site situated between an asparagine and a glutamine residues. Analysis of the amino-acid-residues sequence revealed the presence of a cell wall anchoring motif (LPXTGX) (SEQ ID NO: 14) located ~~between~~ between residues 974 and 981.

The last paragraph bridging pages 26 and 27 has been amended as follows:

To confirm the presence by PCR amplification of BVH-P7 (SEQ ID NO :1) gene, the following 4 serologically distinct *S. pyogenes* strains were used: the serotype M1 *S. pyogenes* strain ATCC700294 and the serotype M3 *S. pyogenes* strain ATCC12384 were obtained from the American Type Culture Collection (Manassas, VA) (~~Rockville, MD~~); the serotype M6 *S. pyogenes* SPY67 clinical isolate was provided by the Centre de recherche en infectiologie du Centre hospitalier

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de l'université Laval, Sainte-Foy; and *S. pyogenes* strain B514 which was initially isolated from a mouse was provided by Susan Hollingshead, from University of Alabama, Birmingham. The *E. coli* strain XL1-Blue MRF' was used in these experiments as negative control. Chromosomal DNA was isolated from each *S. pyogenes* strain as previously described (Jayarao BM et al. 1991. J. Clin. Microbiol. 29:2774-2778). BVH-P7 (SEQ ID NO :1) gene was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from the genomic DNA purified from the 4 *S. pyogenes* strains, and the control *E. coli* strain using the oligonucleotide primers DMAR293 and DMAR294 (Table 1). PCR was performed with 30 cycles of 45 sec at 95°C, 45 sec at 50°C and 2 min at 72°C and a final elongation period of 7 min at 72°C. The PCR products were size fractionated in 1% agarose gels and were visualized by ethidium bromide staining. The results of these PCR amplifications are presented in Table 2. The analysis of the amplification products revealed that BVH-P7 (SEQ ID NO :1) gene was present in the genome of all of the 4 *S. pyogenes* strains tested. No such product was detected when the control *E. coli* DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

The last paragraph bridging pages 32 and 33 has been amended as follows:

Sera collected from eight mice immunized with BVH-P7 His-tagged recombinant protein were analyzed by cytofluorometry and the results are presented in Table 4. All of the sera collected from mice immunized with purified BVH-P7 His-tagged protein contained BVH-P7-specific antibodies that efficiently recognized their corresponding surface exposed epitopes on the heterologous (ATCC12384; serotype M3) *S. pyogenes* strain tested. The fluorescence index varied from 10 to 18. It was determined that more than 97 % of the 10,000 *S. pyogenes* cells analyzed were labeled with the antibodies present in the BVH-P7 specific anti-sera. These sera were also pooled and reacted with the following *S. pyogenes* strains: serotype M1 *S. pyogenes* strain ATCC 700294, serotype M3 and serotype M18 *S. pyogenes* strain ATCC12357 were obtained from the American Type Culture Collection (Manassas, VA) (~~Rockville, MD, USA~~); the serotype M6 *S. pyogenes* SPY69 and M2 *S.*

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pyogenes SPY68 clinical isolates were provided by the Centre de recherche en infectiologie du Centre hospitalier de l'université Laval, Sainte-Foy. The BVH-P7-specific antibodies present in the pool of sera collected after immunization with the purified His-tagged recombinant BVH-P7 protein attached at the bacterial surface of each of these streptococcal strains with fluorescence index between 4 up to 9. On the contrary, no labeling of the streptococcal cells were noted when pools of sera collected from unimmunized or sham-immunized mice were used. These observations clearly demonstrate that the BVH-P7 protein is accessible at the surface where it can be easily recognized by antibodies. Anti-S. pyogenes antibodies were shown to play an important role in the protection against S. pyogenes infection.